

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 September 2001 (07.09.2001)

PCT

(10) International Publication Number
WO 01/64023 A1

- (51) International Patent Classification⁷: **A01H 5/00**, (74) Agents: **WEISER, Gerard, J. et al.**; 1600 Market Street, Suite 3600, Philadelphia, PA 19103-7286 (US).
C12N 5/04, 15/29, 15/31, 15/52, 15/82
- (21) International Application Number: **PCT/US01/06275** (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (22) International Filing Date: 28 February 2001 (28.02.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/186,308 2 March 2000 (02.03.2000) US
60/208,763 4 June 2000 (04.06.2000) US
60/257,406 22 December 2000 (22.12.2000) US
60/259,154 29 December 2000 (29.12.2000) US
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicants (for all designated States except US): **AUBURN UNIVERSITY** [US/US]; 309 Samford Hall, Auburn University, AL 36849 (US). **UNIVERSITY OF CENTRAL FLORIDA** [US/US]; 4000 Central Florida Boulevard, Orlando, FL 32816 (US).
- (72) Inventor; and
- (75) Inventor/Applicant (for US only): **DANIELL, Henry** [US/US]; 1255 Marina Point #315, Casselberry, FL 32707 (US).
- Published:
— with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **MARKER FREE TRANSGENIC PLANTS: ENGINEERING THE CHLOROPLAST GENOME WITHOUT THE USE OF ANTIBIOTIC SELECTION**

(57) Abstract: The present invention provides for a method to circumvent the problem of using antibiotic resistant selectable markers. In particular, target plants are transformed using a plastid vector which contains heterologous DNA sequences coding for a phytotoxin detoxifying enzyme or protein. The selection process involves converting an antibiotic-free phytotoxic agent by the expressed phytotoxin detoxifying enzyme or protein to yield a nontoxic compound. The invention provides for various methods to use antibiotic-free selection in chloroplast transformation.

WO 01/64023 A1

PCT/US01/06275